Vol. XI. *July, 1906.* *No. 2*

BIOLOGICAL BULLETIN

ON THE INFLUENCE OF ELECTROLYTES UPON THE RATE OF RHYTHMIC MUSCULAR CONTRACTIONS (PRELIMINARY COMMUNICATION).

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1. In a previous paper[1] I have suggested that the rate of beat of a rhythmically contracting tissue, such as the heart, may be connected with the velocities of the ions in the tissue-fluids, or in the medium in which the heart is suspended, by the following formula:

$$t = a \cdot \frac{u}{v(u + v)} + b,$$

where t is the interval between the beginning of one beat and the beginning of the next, u is the average velocity of the kations in the medium, v that of the anions in the medium and a and b are constants for any given heart.

2. The assumptions involved in the deduction of this formula are: (1) That the ions diffuse into the tissue in numbers proportional to their migration-velocities in water; (2) that they give rise to a reversible chemical change in the tissue, a change consisting in the displacement of ions already present in the tissue in ion-proteid compounds by those which have newly entered, and that this reaction obeys Guldberg and Waage's mass-law and (3) that the ions thus displaced in each successive segment of tissue give rise to the contraction.

[1] *Pflüger's Arch. f. d. ges. Physiol.*, Bd. 110, p. 610.

3. It occurred to me that, provided the above-mentioned assumptions were correct, the efficacy of a saline medium in maintaining the heart-beat at a normal rate should depend upon two factors, namely, the reversibility of the chemical changes inaugurated by it, and the value of $u/[v(u+v)]$ for the solution. Any solution bringing about only reversible changes (that is, for example, not containing heavy metals) and having the same value of $u/[v(u+v)]$, as Ringer's solution, should be equally efficacious in maintaining the heart-beat. Loeb has introduced the conception of Physiologically Balanced Salt Solutions.[1] It may be that as far as rhythmically contracting tissues are concerned the value of $u/[v(u+v)]$ may afford us a clue to the theoretical composition of such solutions.

4. I made up a Ringer solution with the following composition: $N/10$ NaCl + $N/250$ KCl + $N/200$ $CaCl_2$, which proved to be a good heart solution. The NaCl was made free from potassium by volatilizing the KCl out of Kahlbaum's C.P.NaCl, KCl having a lower boiling-point than NaCl. The average value of u for such a solution can be found by multiplying the velocity of each kation by its equivalent molecular concentration, and dividing the sum of these products by the sum of the concentrations. The value of $u/[v(u+v)]$ for this Ringer solution, calculated from the values for the ionic velocities given in Landolt-Börnstein,[2] is 621.2×10^{-5}. A solution of $LiNO_3$, NH_4NO_3 and $NaNO_3$ having approximately the same value for $u/[v(u+v)]$ is one made up as follows: $.135N$ $LiNO_3$ + $.03N$ NH_4NO_3 + $.01N$ $NaNO_3$—the value of $u/[v(u+v)]$ (calculated from the table of ionic velocities quoted above) is 628×10^{-5}. This forms an excellent heart solution; twenty-three variations of this solution were tested, keeping the concentration of the $LiNO_3$ constant, and varying that of the NH_4NO_3 and $NaNO_3$. In solutions 1–5 the $LiNO_3$ and $NaNO_3$ were kept at $.135N$ and $.015N$ respectively, and the NH_4NO_3 varied from $.03N$ to $045N$, increasing in concentration in each solution from 1 to 5 by $.005N$. In solutions 6–9 the concentrations of the $LiNO_3$ and NH_4NO_3 were kept at $.135N$

[1] *American Journal of Physiology*, Vol. III. (1900), p. 431, "Studies in General Biology," Decennial Publications of the University of Chicago, Vol. II., p. 590.

[2] *Physikalische-Chemische Tabellen*, 1905.

and .03N respectively, and that of the $NaNO_3$ was raised from 0.15N to .035N, differing by .005N in each successive solution.

Solution 10 was the calculated solution. In solutions 11–15 the concentrations of the $LiNO_3$ and $NaNO_3$ were kept at .135N and .015N respectively, while the concentration of the NH_4NO_3 was decreased from .02N in 11 to .000N in 15 by steps of .005N. In solutions 16–19 the concentrations of the $LiNO_3$ and $NaNO_3$ were kept at .135N and .015N respectively, while that of the NH_4NO_3 was raised from .031N in 16 to .034N in 19 by steps of .001N. In solutions 20–23 the concentrations of the $LiNO_3$ and NH_4NO_3 were kept at .135N and .03N respectively, and the concentration of the $NaNO_3$ raised from .016N to .019N in steps of .001N. Out of all the twenty-three solutions thus tried the only ones which approximated to the Ringer as sustainers of the heart-beat were the calculated solution (10) and solution 1 (.135N $LiNO_3$ + .03N NH_4NO_3 + .015N $NaNO_3$) in which $u/[v(u + v)] = 629.5$ — and these are the two solutions which approximate most closely to Ringer in the value of $u/[v(u + v)]$. Thus solution 11 (.135N $LiNO_3$ + .02N NH_4NO_3 + .015N $NaNO_3$) was much less efficacious than the calculated solution. On estimating the value of $u/[v(u + v)]$ for this solution the reason becomes clear, for its value is 615.1, a value departing comparatively widely from that for Ringer or for solution 10. The other solutions in which the NH_4NO_3 concentration was still further lowered (11–15) were still more deleterious. Raising the concentration of the $NaNO_3$ above that in solution 1 was also deleterious (solutions 6–9), and it will be found that in these solutions the value of $u/[v(u + v)]$ rises above 630, as might be expected; raising the concentration of the NH_4NO_3 (solutions 1–5) was still more deleterious.

In solution 10, to quote one experiment, a heart washed in NaCl and beating initially at a rate of 10 in 15 seconds, continued beating for 65 minutes; in 1, a heart beating initially at the same rate and treated in the same way as the above, and at the same time, beat for from 40–60 minutes; while a third heart, in Ringer, and beating initially at the same rate as the other two, beat for 90 minutes.

5. Thinking that perhaps the concentrations of these solutions were somewhat too high to be altogether favorable, I made up a series of six solutions with the concentration of $LiNO_3$ in each $.1N$ — one, solution 24, was calculated so as to give a value of $u/[v(u+v)]$ approximating to that in Ringer — its composition was $.1N LiNO_3 + .02N NH_4NO_3 + .015N NaNO_3$, and the value of $u/[v(u+v)]$ was 626.7. The remainder were solutions containing the same concentrations of $LiNO_3$ and $NaNO_3$, but varying in NH_4NO_3 concentration from $.02N$ (24) to $.025N$ (29). The most favorable solution was 24, the others departing more widely in the value of $u/[v(u+v)]$ from that for Ringer.

This new calculated solution formed a really excellent sustainer of the heart-beat, and by the addition of a trace (1 c.c. $N/40$ to 100 c.c. solution) of Na_2CO_3, to give a favorable alkalinity, a solution was obtained which was in every way as favorable a medium for the heart-beat as the Ringer to which the same amount of Na_2CO_3 had been added. Frogs' hearts beat in these solutions (24 + Na_2CO_3 and Ringer + Na_2CO_3) for over two hours.

6. Thus the facts are in favor of the hypothesis, and we see that neither K, Ca or Cl ions are specific and essential for the heart-beat, nor, probably, Na ions — all that is necessary is to obtain a properly balanced solution containing no ions (such as heavy metals) which make irreversible compounds with proteids, and having the most favorable value of $u/[v(u+v)]$. Such a solution could be made up from any two or three salts whose values of $u/[v(u+v)]$ differed sufficiently widely in opposite directions from the value for Ringer. It is not inconceivable, of course, that a favorable heart solution may ultimately be found which contains a heavy metal — but one would not expect to find such a solution having the same value of $u/[v(u+v)]$ as Ringer, since the constants a and b (in the equation which I have quoted) would probably be altered by the heavy metal.

These experiments will be continued.

7. In the course of an investigation on the influence of temperature upon the rate of the heart-beat in *Ceriodaphnia* the results of which have appeared in the BIOLOGICAL BULLETIN,[1] I found that the rate of the heart-beat in *Ceriodaphnia* is practically constant

[1] Vol. X., p. 242 (1906).

at 21° C., provided the different individuals are taken from the same vessel, treated in the same way, and not previously exposed to any extreme temperatures. With these precautions individuals differing widely in development will be found to have practically the same rate of heart-beat at 21°. *Ceriodaphnia* can therefore be used as homogeneous material for the investigation of the influence of electrolytes upon the rate of the heat-beat — that is, the constants a and b in the equation

$$t = a \cdot \frac{u}{v(u+v)} + b$$

are practically the same for all the individuals, and the influence of each solution can be tested upon a fresh individual. The number of beats was registered upon a revolving drum by tapping a key in a circuit containing a signal-magnet, and the time was taken with a stop-watch.

8. The following are the experimental results so far obtained, the "time of one beat" meaning the interval between the beginning of one beat and the beginning of the next. The values of $u/[v(u+v)]$ are calculated from the table of ionic-velocities to which I have previously referred. In every case the organisms were first washed in tap-water, then placed in about 50 cc. of the solution for 10 minutes and then placed, with a drop of solution, on a glass plate kept accurately at 21° by means of a stream of water running underneath — in 10 minutes the rate of beat was noted.

Solution.	$u/[v(u+v)]$	Time of one beat.	Value of a when $b=1.7432$.
$N/10$ $LiNO_3$	567.7×10^{-5}	.3 sec.	356.4
$N/10$ NaCl	611.6×10^{-5}	.150 "	306.6
Ringer	621.2×10^{-5}	.183 "	30 .9
100 c.c. Ringer + 2 cc. $N/10$ KCl	624.1×10^{-5}	.185 "	305.75
$N/10$ $MgCl_2$	631.4×10^{-5}	.2 "	304.6
40 c.c. $N/10$ NaCl + 10 c.c. $N/10$ KCl	645.9×10^{-5}	.238 "	303.7
30 c.c. $N/10$ NaCl + 20 c.c. $N/10$ KCl	677.8×10^{-5}	.353 "	306.6
30 c.c. $N/10$ $LiNO_3$ + 20c.c. $N/10$ NH_4CNS	725.1×10^{-5}	.375 "	289.4
$N/10$ NH_4CNS	937.6×10^{-5}	.5 "	237.1

Thus we see that the value of a is constant for values of $u/[v(u+v)]$ between and including 611.6×10^{-5} and 677.8×10^{-5}. Above and below those values it is variable.

9. It is evident that upper and lower limiting values of $u/[v(u+v)]$ exist between which sustained beats are possible.

The existence of the lower limit is evident from a consideration of the equation

$$t = \frac{au}{v(u+v)} + b,$$

for since b has a negative sign, when

$$\frac{u}{v(u+v)} = -\frac{b}{a}$$

then the time of one beat is zero, and for still lower values of $u/[v(u+v)]$ the time of one beat would be negative, which is, of course, impossible. The equation

$$\frac{u}{v(u+v)} = -\frac{b}{a},$$

therefore gives us a theoretical lower limit, in this case 562×10^{-5}. Before this, however, as the experiment with $LiNO_3$ shows, the beats begin to fuse — for the beats take a certain time to traverse the heart, and this interval may be greater than that between the beginnings of two beats. The upper limit is possibly the expression of the fact that ions must reach the minimum number necessary to displace those in combination with the proteids (the "threshold-number") before they have time to diffuse away through the tissue. Before this point, however, irregularities appear, as in the last two experiments in the table above. The beats soon stop in these extreme solutions. Thus in KCl or in 20 c.c. $N/10$ NaCl + 30 c.c. $N/10$ KCl the beats are only sustained for about 5 minutes at 21° — in the latter solution the value of $u/[v(u+v)]$ is 706.9, and we notice that it is just about at this value of $u/[v(u+v)]$ that irregularities begin to appear in the value of the constant a. Hence the limits for *Ceriodaphnia* are probably 570×10^{-5} and 700×10^{-5}. Any solution in which $u/[v(u+v)]$ lies between these values, and which does not contain any substance forming irreversible proteid compounds, should sustain the heart-beat in this organism — solutions having values of $u/[v(u+v)]$ falling outside these limits could not sustain the heart-beat.

10. By mixing a solution having a value of $u/[v(u+v)]$ below the lower limit for the rhythmically contracting tissue in question

with one having a value of $u/[v(u + v)]$ above the upper limit, a solution could evidently be obtained which would sustain the heart-beat. In this way many salt-antagonisms could be obtained. Thus I have found that the heart of *Ceriodaphnia* will not beat in $N/10$ KCl or in 20 c.c. $N/10$ NaCl + 30 c.c. $N/10$ KCl, but will beat in 30 c.c. $N/10$ NaCl + 20 c.c. $N/10$ KCl, because the two former solutions have values of $u/[v(u + v)]$ lying above the upper limit for this rhythmically beating tissue, while the last-mentioned solution has a value of $u/[v(u + v)]$ lying between the upper and lower limits. If we were considering the question as one of toxicity, we should say that NaCl *antagonized* KCl. The possible reason for the antagonism is now clear.

11. The values of the upper and lower limits for rhythmical contractions in a tissue will obviously depend upon the constants a and b for that tissue in the equation

$$t = a \cdot \frac{u}{v(u + v)} + b.$$

These constants will be different in different tissues, and need not be the same for different parts of the same tissue. Thus, in the case of *Gonionemus*, it is only necessary to suppose that the limits for the center lie very close on either side of the value of $u/[v(u + v)]$ for NaCl, while the limits for the margin are somewhat farther apart, to obtain a possible explanation of the fact that the center will beat in NaCl solution but not in NaCl solution to which KCl or $CaCl_2$ have been added, while the *margin* will continue to beat in such solutions.[1] In this way many of the apparently specific effects of certain ions upon rhythmically contracting tissues may possibly be reduced to a general explanation.

12. Experiments will be continued with a view to obtaining further evidence as to the existence of upper and lower limits in the value of $u/[v(u + v)]$, above and below which rhythmic contractions are impossible, and also further experiments upon the degree to which the crustacean heart obeys the formula

$$t = a \cdot \frac{u}{v(u + v)} + b.$$

[1] Loeb, *American Journal of Physiology*, Vol. III. (1900), p. 383.

THE EYES OF THE BLIND VERTEBRATES OF NORTH AMERICA.

VII. The Eyes of Amphisbæna punctata (Bell), A Blind Lizard From Cuba.[1]

FERNANDUS PAYNE.

Amphisbæna punctata is a blind legless lizard which burrows in the ground. It is common in Cuba to which it is restricted. How deep it burrows, I do not know, but it is often turned out by the plow. The specimens obtained ranged from 103 to 245 mm. in length. The head is short, hard and pointed, and the tip of the upper jaw projects slightly beyond the tip of the lower jaw. In shape, arrangement of the dermal plates, and in the color of the ventral surface of the body it closely resembles an earthworm. The dorsal surface is flesh-color with small brown spots. The tail is short and flattened dorsoventrally. In a specimen 245 mm. in length, there were 225 annuli on the dorsal side, 202 on the ventral and 15 on the tail. In this specimen the tail was one thirteenth and the head one thirty-fifth the length of the body.

Methods. — The lizards were put alive into formalin. They were afterwards changed to alcohol. For decalcification, the heads were placed in five per cent. nitric acid from twenty to thirty days. A shorter period did not give satisfactory results. Some of the heads were imbedded in paraffin and others in paraffin and celloidin. In using the latter method I imbedded the head in celloidin in the usual manner and hardened in chloroform. From chloroform I transferred the block to soft paraffin for twenty-four hours and thence to hard paraffin for twenty-four hours, after which I imbedded the block in paraffin.

[1] Contribution from the Zoölogical laboratory of Indiana University, No. 77. The material used in the preparation of this paper was incidentally collected during several expeditions to Cuba. The prime object of the expeditions was to collect life history material of the Cuban blind fishes, *Lucifuga* and *Stygicola*. They were undertaken with a grant of $1,000 from the Carnegie Institution.

The best results were obtained from those imbedded in paraffin and celloidin. Several methods of staining were used; iron hæmatoxylin with eosin as a counterstain gave the best results. The more modern methods of treating the retina with silver could not be applied for lack of fresh specimens. On account of the extreme toughness of the cuticle it was impossible to get complete series of sections. For comparison I have examined the eye of *Anolis carolinensis*.

General Account of the Eye.

The eye of *Amphisbæna* appears indistinctly as a small black spot beneath the ocular plate (Fig. 1). In a specimen 225 mm. in length, the eye is 352 μ beneath the surface, 420 μ in width and 360 μ in depth. The conjunctival sac is 116 μ in diameter. The conjunctiva is very thin over the cornea, but measures 4 μ in thickness over the anterior part of the sac.

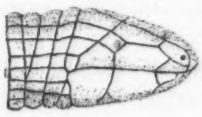

Fig. 1.

The dermis and epidermis have the same structure over the eye as over the regions near by. This corresponds with what Eigenmann ("The Eyes of *Rhineura floridana*," 1902) found in *Rhineura*, although the eye of *Rhineura* is a much more degenerate organ than the eye of *Amphisbæna*. To what extent the eye has degenerated from a more elaborate structure I am unable to say. Few organs are stationary and this one is probably still in process of reduction. I have been unable to obtain the young, and there is no means of finding out from the adult whether the eye is degenerating at present or not. In each specimen examined the eyes appeared in about the same state of degeneration.

The eye measures 1,224 μ in circumference and the pupil 104 μ in diameter. The uveal part of the iris on each side of the pupil measures 250 μ. The pupil and iris occupy 49.3 per cent. or very nearly half of the entire circumference.

Harder's gland is very much larger than the eye. In a cross-section through the central portion of the eye, the antero-posterior diameter is approximately three times and the medio-lateral diameter four times the medio-lateral diameter of the eye. It is

divided into two distinct lobes, the anterior being much smaller than the posterior. The gland completely surrounds the eye except over the anterior face. Its secretion is poured into the conjunctival sac and from thence into the mouth cavity. The large size of the gland in *Typhlops* led Duvernoy to the conclusion that its function was not connected with the eye. As its secretion, in *Amphisbæna*, is poured into the conjunctival sac and thence into the mouth cavity, its function must have been, primarily at least, connected with the eye.

No eye muscles are present in *Amphisbæna*.

The eye is directed outward and forward and makes an angle of about 60° with a line drawn tangent to the dermal plate which covers it.

Whether the eye is still used as a sense organ, I cannot say, but since the parts are so well developed and the eye is not buried very deeply beneath the surface, I am inclined to believe that it is at least, susceptible to light.

The Sclera. — The sclera (*scl.*, Fig. 6) has apparently undergone no degeneration whatever. It compares favorably with that of *Anolis*. In fact, there is but little difference in its structure in the two eyes. At the proximal part of the eye, the sclera measures 12 μ in thickness, while at the same place in *Anolis* it measures 15 μ. It is continuous over the front of the lens as the cornea, which together with the thin wall of the conjunctival sac at this place measures 7 μ. Scleral cartilages extend from about the middle of the eye back almost to the optic nerve. On each side of the sclera and forming a part of it, are thin irregular layers of pigment in patches.

The Choroid. — If the blood vessels in the choroid still persist, the preparations do not show them. All that can be seen is a number of densely pigmented cells, around and between which are filaments of connective tissue (*chr.*, Fig. 6). At the entrance of the optic nerve, this layer measures 8 μ in thickness, but gradually becomes less forward and vanishes entirely a short distance back of the enlarged end of the pigment layer. The pecten, present in *Anolis*, is not apparent in *Amphisbæna*.

The Lens. — The lens has retained its natural shape and position (*lens*, Fig. 6). It is almost spherical and measures 80 μ in

diameter. In most of the sections an outer layer of cells extends around the anterior surface of the lens. The interior in nearly every case stained as a structureless mass, but in a few sections it appeared to be made up of large irregularly shaped cells with small nuclei. If any fibrous cells still persist, they did not show. No capsule is present.

The Vitreous Body. — The vitreous body (*vit. cav.*, Fig. 6) occupies the greater part of the eye-ball and has certainly undergone but little change. The aqueous cavity has entirely disappeared.

The Iris. — Only the uveal part of the iris remains. It is continuous with the pigment epithelium of the retina and has the same structure. In the thickest part it measures 68 μ. The cells are similar to those of the pigment layer, except that their radial diameter is much greater. The ciliary processes are no longer present.

The Optic Nerve. — The optic nerve can be traced from the eye, through and along the side of Harder's gland. While the nerve could be traced no further on account of an incomplete series of sections, there is no doubt that the connection with the brain still exists. The nerve fibers enter the eye in a compact mass, pass through the layers of the retina until they reach the nerve fiber layer, where they spread out and connect with the nerve cells of the ganglionic layer in the usual manner.

The Retina.

While the retina has undergone considerable change, all the layers are still present (Fig. 3). It measures 78 μ in thickness. In *Anolis* about half way between the anterior and posterior parts of the eye it is 179 μ in thickness. If the macula lutea is still present, the preparations do not show it.

The Pigment Layer. — The pigment layer (1, Fig. 6) which bounds the retina externally, consists of a single stratum of rectangular cells separated by a small amount of clear intercellular substance. These cells have large oval nuclei free from pigment, almost transparent and with small nucleoli. At the back portion of the eye where the pigment layer measures 8 μ, the transverse diameter of the cells is greater than the radial diameter, but

toward the anterior portion where the layer becomes thicker, the radial diameter becomes much the greater. The greatest thickness of this layer is near the lens, where it measures 68 μ. The outer surface of the pigment cells — that which lies next to the choroid — is smooth and slightly convex. The inner surface, on the other hand, is very irregular. The cells at this place are very densely laden with pigment and prolonged into filamentous processes which extend between and amongst the cones. In fact, the cones may be said to be imbedded in the pigment cells. This layer differs but little from that of *Anolis*, except at the anterior part of the eye where it becomes much thicker.

The Cones. — No rods are present. The cones (2, Fig. 3) consist of an upper and a basal portion. The basal part is elliptical in shape and stains uniformly throughout, while the outer portion is longer and somewhat triangular in shape, with the smaller side of the triangle resting on the inner elliptical part. This layer measures 10 μ in depth while the same layer in *Anolis* measures 13 μ.

The Outer Nuclear Layer. — This layer is made up of a single stratum of nuclei with small dark nucleoli (3, Fig. 3). Some of these nuclei are almost spherical, while others are oval in shape. They are connected with the cones by broad processes which stain darkly. These processes may be very short, in which case the cone comes in close proximity to the nucleus; or they may be drawn out into filaments as long as or longer than the nuclei themselves. From the inner part of the nuclei extend processes which broaden toward the base and send numerous ramifications into the inner stratum of the outer reticular layer. There is a striking difference here between this eye and the normal one. The processes from the base of the nuclei pass straight through the outer reticular layer while in my sections of the normal eye, they pass through at an angle of about 45° (3, Fig. 4).

The Outer Reticular Layer. — The outer reticular layer (4, Fig. 3), is penetrated by the processes from the nuclei of the outer nuclear layer and by a few Müllerian fibers. If processes from horizontal cells are present they were not brought out by the method of staining which was used. Again, there is but little

difference in the thickness of this layer in the two eyes, as it measures 6 μ in *Amphisbæna* and 7 μ in *Anolis*.

The Inner Nuclear Layer. — The inner nuclear layer is a compact mass of somewhat irregular spherical nuclei and is 24 μ in thickness (6, Fig. 3). The corresponding layer in *Anolis* is 59 μ. Spongioblast and bipolar cells cannot be differentiated from each other. All the nuclei appear to be very much alike, except the nucleated enlargements of the fibers of Müller, which have no definite shape and which stain very densely. However, some nuclei, more especially those of the inner stratum, stain a very deep black color, and show no structure whatever. Parts of certain other nuclei stain densely, while the rest retains its original identity. Some of the nuclei have from four to six nucleoli. In *Anolis* two other kinds of nuclei appear. A few flattened horizontal nuclei can be seen near the middle of the layer and in the inner stratum are a number of large spherical nuclei. Penetrating this layer are many fibers of Müller. Each fiber as it passes through is characterized by a nucleated enlargement.

The Inner Reticular Layer. — The inner reticular layer measures 20 μ in thickness as against 45 μ in *Anolis* (8, Figs. 3 and 4). The method of staining brought out no definite structures. The fibers of Müller pass through it as fine vertical filaments. Occasionally there is a nucleus from the nuclear layer or from the ganglionic layer which lies imbedded in the edge of this layer.

The Ganglionic Layer. — The ganglionic layer (9, Fig. 3) consists of a single layer of nuclei 6 μ in diameter, with now and then another nucleus above or below the single layer. From the outer side of these nuclei fibers which run out and penetrate the inner reticular layer, can be traced for a short distance. On the opposite side are also fibers which continue as fibers of the nerve fiber layer. In *Anolis* this layer measures 23 μ and is made up of loosely connected nuclei, some of which are large and spherical, others are smaller and irregular, while still others stain very densely.

The Nerve Fiber Layer. — The nerve fiber layer is 6 μ in depth while in *Anolis* it is 26 μ.

The Fibers of Müller. — The Müllerian fibers can be traced from the membrana limitans interna to the outer nuclear layer.

They commence at the inner surface of the retina by a broad conical foot which extends into the ganglionic layer. Through the inner reticular layer the fibers pass as fine filaments, but in the inner nuclear layer each fiber is characterized by an irregularly shaped nucleus, which stains densely and shows no structure. The membrana limitans externa is not visible. These fibers differ but little from those in *Anolis*, except that those in *Anolis* can be traced to the membrana limitans externa, which is plainly visible.

Summary.

1. The eye muscles have entirely disappeared.

2. Only the uveal parts of the iris remain.

3. The lens has retained its shape and position, but its structure has been greatly changed. No capsule is present.

4. Harder's gland is many times larger than the eye and pours its secretion into the conjunctival cavity and thence into the mouth.

5. The sclera, scleral cartilages, cornea, vitrous body and pigment epithelium have undergone but little change unless it be in the reduction in size.

6. The cuticle passes over the eye unchanged.

7. The aqueous cavity is no longer present.

8. All the layers of the retina are still present. As shown in Fig. 6, the great reductions in the depth of the layers, in comparison with those of *Anolis*, has taken place in the nerve fiber, ganglion cell, inner reticular and inner nuclear layers.

9. If the eye has been reduced from an eye of the average size, all parts have certainly undergone considerable change, and this change has been approximately equal among the several parts.

10. The retina does not show such a profound change as either the iris, muscles or lens. However, it has been greatly changed, as it extends only 50.7 per cent. of the distance around the eye.

11. The eye of *Amphisbæna* bears out the statement made by Eigenmann ("Eyes of the Blind Vertebrates of North America," I.) that the more active parts of the eye are the ones to degenerate first. They are the parts which have been most affected.

ACKNOWLEDGMENTS.

I took up the work on this lizard at the suggestion of Dr. C. H. Eigenmann and it is under his direction that the work has been carried forward. To him I am indebted for many helpful suggestions and for my specimens. I also wish to express my thanks to Mr. Leonard Haseman, of Lake City, Florida, for sending me specimens of *Anolis*.

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Explanation of Plate I.

1, pigment layer; 2, cones; 3, outer nuclear layer; 4, outer reticular layer; 6, inner nuclear layer; 8, inner reticular layer; 9, ganglion cell layer; 10, fiber layer; *lens*, lens; *scl.*, sclerotic; *chr.*, choroid; *cor.*, cornea; *scl.c.*, scleral cartilage; *n.op.*, optic nerve; *vit.cav.*, vitreous cavity; *con.cav.*, conjunctival cavity; *C.*, outer covering of the eye; *M.*, Müllerian fiber; *L.*, membrana limitans externa.

Figs. 1, 2 and 5 were drawn from sections. One-twelfth objective and one-inch eye-piece.

Figs. 4 and 6 are diagrammatic.

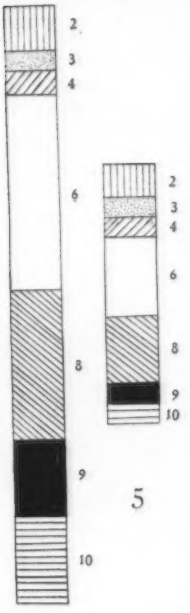
Fig. 1. Side view of the head.

Fig. 2. Diagram of the eye showing the parts in their relation and the distance of the eye beneath the surface.

Fig. 3. Horizontal section of the retina of *Amphisbæna punctata*, showing the different layers.

Fig. 4. Horizontal section of the retina of *Anolis*.

Fig. 5. Diagram showing the comparative measurements of the retina in the two eyes.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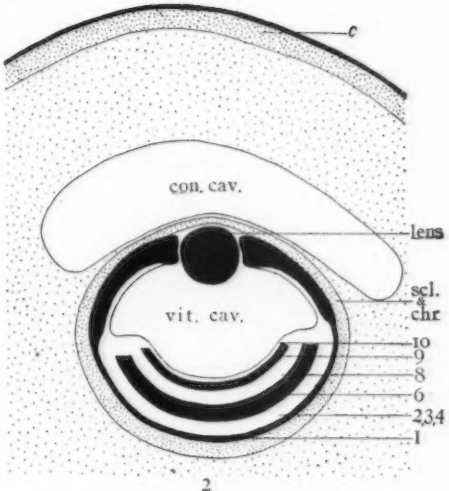
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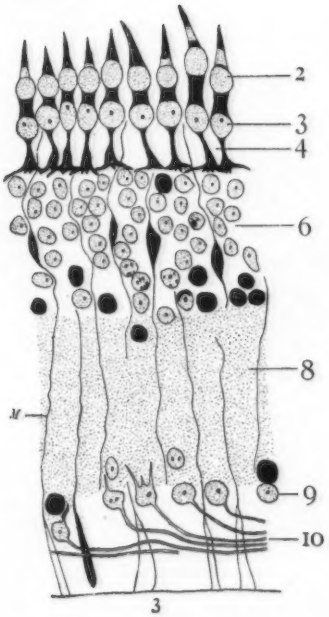
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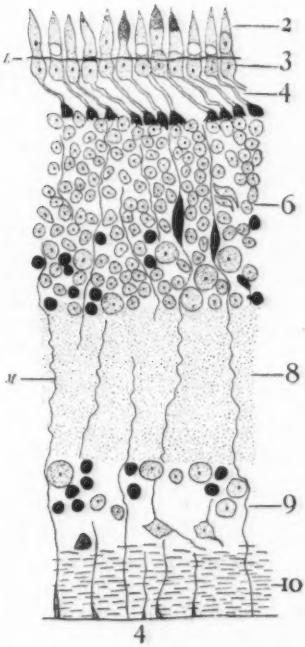
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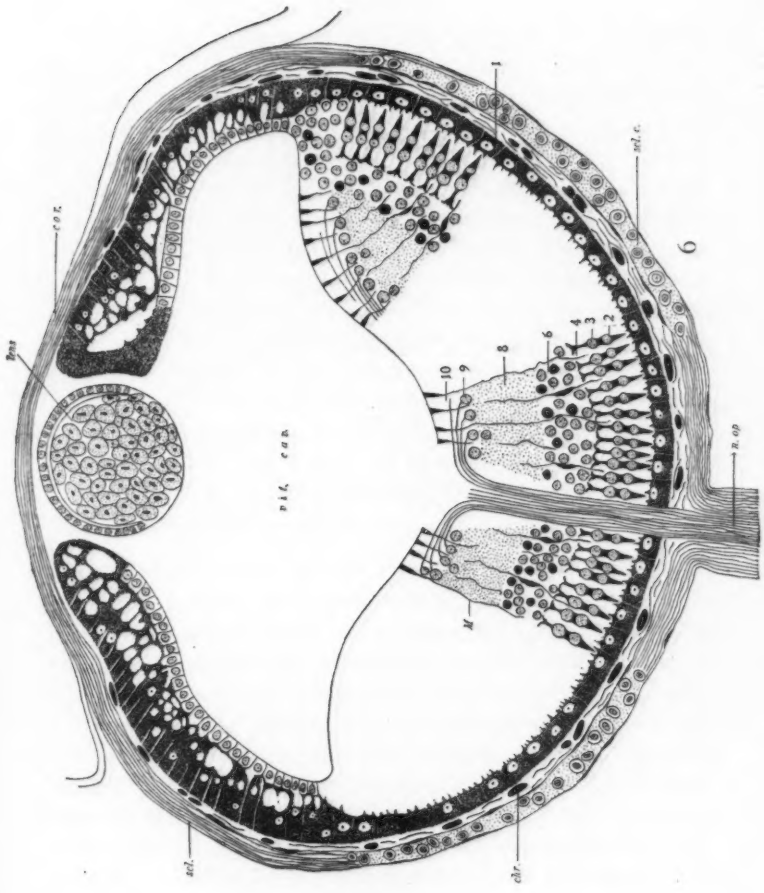
Explanation of Plate II.

Fig. 6. Horizontal section of the eye showing the different parts. The retina is diagrammatic.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EXPERIMENTS WITH FROG'S EGGS.

T. H. MORGAN.

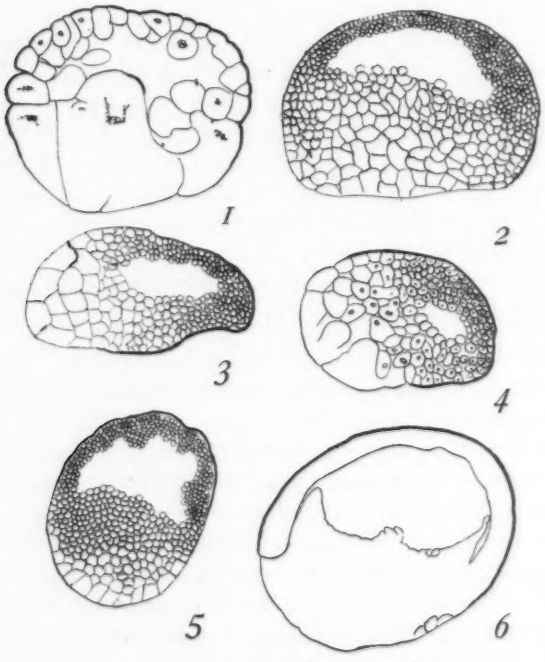
The following experiments were carried out in part during the spring of 1905, and in part during the present year. As the headings of the different sections indicate I have brought together the results of experiments of very different kinds, but since they all bear on the same questions it seemed preferable to put the results together in one paper, rather than to scatter them through several. The following topics are discussed: (1) The Development of the Frog's Egg out of Water, (2) The Increase in the Size of the Egg during the Segmentation Stages, (3) Obliteration of the Blastocœl by means of a Centrifugal Force and the Effect on the Subsequent Development, (4) Removal of the Roof of the Blastocœl, (5) Effects of Cold on the Early Development, (6) The Early Development of the Lithium Larvæ of the Frog, (7) Effects of Lithium Chlorid and Sodium Chlorid Acting Together, (8) Effects of Lithium Chlorid and Magnesium Chlorid Acting Together, (9) The Chemical Versus the Osmotic Effects of Salt Solutions.

The Development of the Frog's Egg Out of Water.

In order to determine whether the segmentation cavity of the frog's egg is simply a water-filled space, left by the cells of the blastula as they separate, or whether the cavity is formed by the active secretion of the surrounding cells, I placed eggs on pieces of filter paper and allowed them to develop out of water. On the first assumption, the blastocœl is filled with water, that, percolating *between* the cells, passes into the interior. The enlargement of the normal egg during the cleavage period shows that water is really absorbed by the egg, but whether this water simply fills the enlarging segmentation cavity, or whether it enters the cells could only be determined by keeping the eggs out of water during the early development.

In the first experiment the eggs were placed on pieces of wet filter paper after their outer membranes had been removed. The

lower end of the paper simply dipped into water. Under these conditions the eggs developed normally and young tadpoles appeared. The eggs may have absorbed enough water from the filter paper for their normal development. In later experiments, the eggs, freed from their outer membranes, were placed on dry pieces of filter paper, six to eight on each small piece. The water adhering to the inner membranes made a small damp spot around each egg. The pieces of paper were then put into glasses with covers to prevent further drying. In the course of a few hours the eggs became flattened on the side in contact with the paper. The segmentation continued under these conditions, and



FIGS. 1–6. Eggs developing out of water.

in a few cases, noticeably those where too much water had been left, normal or abnormal embryos developed. In the majority of cases, however, the egg died, or the development was retarded

in the late cleavage stages, the amount of desiccation determining the result. Despite the drying of the egg with its accompanying flattening, the segmentation cavity appeared in it, as sections showed, and while it generally failed to reach the proportions characteristic of normal development (except when the paper was too wet), yet that it should develop at all under the adverse conditions of the experiment, demonstrates that its contents are produced, in part at least, as a secretion of the surrounding cells. A few sections of these eggs are reproduced in Figs. 1–12.

A section through an egg that had been placed on a dry piece of filter paper at the two-cell stage, and killed some hours later, is shown in Fig. 1. A large segmentation cavity is present in the interior although the egg is flattened below from pressure.

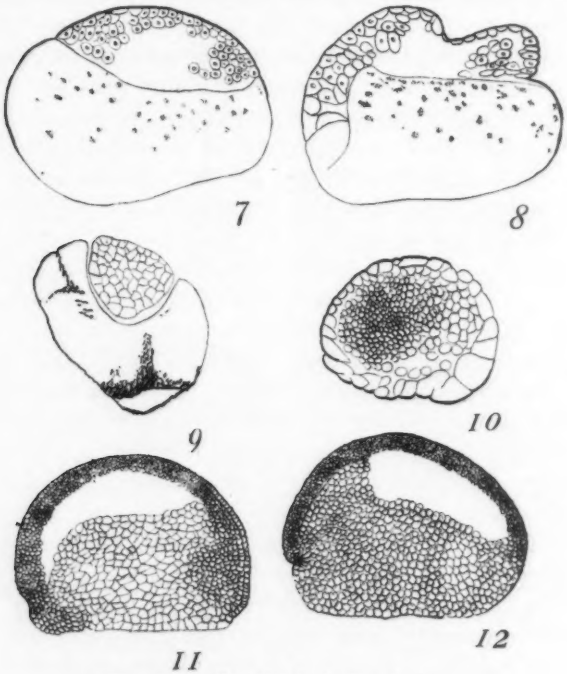
A later stage is shown in Fig. 2. The egg was attached by its white pole. The blastocœl is large and not very different from that of a normal egg. A still later stage of an egg, that was still further compressed (on one side in this case), is shown in Fig. 3. The segmentation cavity is smaller than in the normal blastula of this age. The reduction is probably connected with the great flattening of the egg. Similarly in the next figure, Fig. 4, the segmentation cavity is reduced in size. In the next two figures, Figs. 5 and 6, the segmentation cavity is larger, and as large, in fact, as that of the normal egg. These eggs were but little compressed, and, therefore, developed more nearly in the normal manner.

The next two figures, Figs. 7 and 8, show a different type of development. The yolk has been so much injured by the drying, that it has failed to segment, yet it is filled with nuclei each surrounded by pigment. Both of these eggs were fastened by the lower hemisphere. The tops of the eggs were less injured, and had divided into small cells, that arch over a large segmentation cavity.

The next egg is very different, Fig. 9. Here, too, the yolk has been killed, but there is present a solid mass of cells imbedded in the yolk. The egg seems to have been attached to one side of the black hemisphere, and the small cells in the interior are due to the shifting of the interior of the egg. The next figure, Fig. 10,

shows a somewhat similar condition, where, however, the lighter material of the top of the egg has sunken into the interior. This was due to the eggs being placed on the paper in an inverted position while in the two-cell stage. The figures show that the smallest cells are in the interior of the egg, and not on the surface of the black hemisphere.

In order to meet the possible objection that the egg may absorb water from the small, slightly damp, piece of paper, another series



FIGS. 7–12. Eggs developing out of water.

of experiments was carried out, in which the eggs, deprived of their outer coats, were kept on pieces of glass. For several minutes the eggs were left on the glass exposed to the air, in order that the water sticking to their outer coats, or to the glass, might dry off. They were then placed in the moist chamber. An egg that had reached a late blastula stage under these conditions is

shown in Fig. 11, and another at the beginning of gastrulation in Fig. 12. Both show a large segmentation cavity. The results may be summed up as follows: If the egg in the two- or four cell-stage is taken from the water and kept from drying, it will develop, and the segmentation cavity will be formed, which, although sometimes smaller than the normal, if the egg is very dry, yet its presence under these adverse conditions shows that it must be due to a secretion poured out by the blastula cells, and that it is not due directly to the passage of the water from the outside into the egg.

I have found that the eggs of *Fundulus heteroclitus* will also develop out of water if simply placed on a glass plate in a moist atmosphere. The eggs of the starfish will pass through several segmentation stages under similar conditions, but so little water remains on the glass that it evaporates quickly and the eggs are so delicate that they cannot withstand the drying. The blastula becomes flattened and is nearly solid, but on being placed again in sea water it quickly rounds up, and the segmentation cavity appears. The normal segmentation cavity in the starfish is very large and is early formed. Its absence in the eggs out of water may be due to the flattening of the egg, but possibly its absence is due to the necessity of the cells to absorb water in order that it may be formed. Those who have studied the segmentation of the sea urchin egg in water under compression, as when it is placed between compressing plates, have observed the absence of the segmentation cavity, even when the segmenting egg has become two-layered. In the frog also, as we have seen, the compression may be responsible for the suppression of the full development of the blastocœl, but since the compression is not carried so far in the latter case, the formation of the cavity is not suppressed, and the fact that it forms at all under these adverse conditions goes to show that its origin is due to the activity of the surrounding cells.

The Increase in the Size of the Egg During the Segmentation Stages and the Increase of the Blastocœl.

I measured some eggs of *Rana sylvestris* at different stages of development in order to see whether the increase in size of the

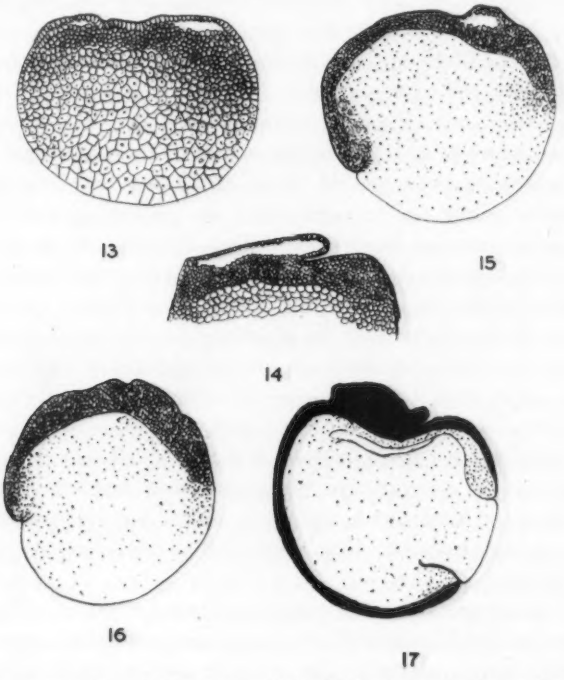
egg during the segmentation stages and pregastrula stages is equal to or greater than the space of the blastocœl. On account of the variation in size of different eggs it would have been better to measure the same egg at different times. I shall give some measurements of several eggs of the same bunch but not the same eggs. In one case the unsegmented eggs measured 5.1, 5.2, 5.3 and 5.4. The average eggs seem about 5.3. At the beginning of gastrulation the eggs measured 5.6 and 5.7. When the dorsal lip was widely horseshoe-shaped the eggs measured 5.6 and 5.8; and when the gastrula lips were nearly close 5.8 and 5.9. In another case the unsegmented eggs measured 5.2, 5.3, 5.4 and at the beginning of gastrulation 5.5, 5.6, 5.7. In another case the unsegmented eggs measured 5.2, 5.3, 5.4, and at the beginning of gastrulation 5.4, 5.5, 5.8.

If we assume that the unsegmented egg measures 5.3 and that the egg about to gastrulate measures 5.6, the latter will have gained about two ninths of the volume of the former, or roughly one fourth. An approximate estimate of the size of the blastocœl when fully formed, as compared with the blastula in which it is contained, shows that the blastocœl is about one eighth the entire volume. Hence, while the egg has gained one fourth in volume, only one eighth of its increase is due to the segmentation cavity; *i. e.*, only one half of the increase in size can be accounted for by the segmentation cavity alone, and the other half must have been due to the absorption of water by the cells of the blastula. Thus we must conclude that the egg is absorbing water during the segmentation stages, and that at the same time it is giving up to the blastocœl an amount of fluid that is approximately half of the amount absorbed. If the egg is placed under conditions where it can not absorb water, it gives up, nevertheless, nearly the normal amount to the blastocœl.

Obliteration of the Blastocœl by Means of a Centrifugal Force and the Effect on Subsequent Development.

The eggs of *Rana sylvestris* in a late blastula stage were put into tubes of water and revolved on a small centrifuge at the rate of 1,600 revolutions per minute for ten minutes. At the end of

this time the black hemisphere of the egg was much flattened, and sections show, Fig. 13, that the fluid of the blastocœl has been completely driven out of the egg. The figure shows that not only has the top of the egg been flattened, but the small cells at the sides of the blastocœl have moved over across the top of the yolk, so that a thick layer of small cells lies as a flat plate on top of the larger yolk cells. Here and there a small crack or space is present along the region of contact of the large and the small cells. Not infrequently the top of the egg is thrown over as a fold, as shown in Fig. 14. This is due no doubt to the fact that as an arch it occupies more space than when flattened down on the egg. In the preserved eggs an artefact often appears beneath the outer layer of cells.



FIGS. 13–17. Eggs of *Rana sylvestris* after having been rotated at 1,600 revolutions per minute. 13, immediately after rotating; 14, top of another egg at same time; 15–17, later stages when gastrulation has begun.

These eggs gastrulated and produced embryos. Sections through several of the intermediate stages show that, after the removal from the machine, the top of the egg did not regain its former roundness, Fig. 15, but remained flattened. In a few cases a small space was found near the top of the egg beneath the ectoderm that may represent a part of the blastocœl, Fig. 16. A later stage is shown in Fig. 17. The blastopore is closing, the archenteron is present, but no indications of the blastocœl can be found. A lump of ectoderm lies near the anterior end of the embryo. It is an almost constant feature of these embryos, and owes its origin to the injury of the roof of the blastocœl. Its location does not necessarily mean that the normal embryo extends to the top of the egg, because extensive movements of the cap of small cells must take place during the time of gastrulation. Nevertheless it is true that this lump of cells originated near the top of the egg, and had been carried downwards towards the anterior end of the embryo. In fact the material of which it is composed may represent the ectoderm of the anterior end that had been carried upwards by the action of the centrifugal force.

The results show that the blastocœl is not essential for the formation of the frog embryo, since the process of gastrulation may take place in its absence. This does not mean that the blastocœl may not be made use of in ordinary development; in fact it is made use of, since the yolk mass is thrown into it, but the result does mean that the blastocœl is not essential for development. Two methods of interpreting the blastocœl have been followed by embryologists. The commonly accepted method is to "explain" it by assigning to it a purpose. Its purpose is to make a space into which the yolk cells can be thrown at the time of gastrulation. It would seem from this point of view that the blastocœl is not only a useful, but also an essential organ in development. The results show, however, that this is not the case.

The other method of interpretation is that of the school of developmental mechanics which has tried to account for the formation of the blastocœl as the results of some such mechanical process as infiltration, and have assigned it to the function of producing an osmotic pressure on the walls of the blastula. Rhumbler

has tried to account for the gastrulation process as the outcome of the accumulation of certain waste products in the blastocœl, but since the development of the gastrula may take place when no blastocœl is present, this explanation of the mechanics of gastrulation does not appeal to me as a probable one.

Removal of the Roof of the Blastocœl.

It has been suggested by Rhumbler that the process of gastrulation may be due to the accumulation of waste products, carbon dioxide for instance, in the blastocœl fluid. The presence of such a substance would bring about changes in the surface tension on one side of the yolk cells, which, by causing them to change shape, is imagined to bring about the inturning of the cells. It seemed to me that this view might be tested by emptying the blastocœl of its fluid before gastrulation had occurred. If the inturning still took place the result would show that the process need not be connected with the blastocœl fluid, or with substances that have become dissolved in it.

There was also another question that I wished to examine by means of the same experiment. The formation of the large blastocœl space takes place at the time when the embryo-forming materials, that come from the upper hemisphere, are moving outwards and downwards at the sides of the blastocœl, and the question arises whether this movement is connected with the development of the blastocœl space. Finally there is still a third question involved, namely, whether the movement of the material is due to the downward pressure of the cells themselves of the roof of the blastocœl.

In the first set of experiments the roof of the blastocœl was opened, and injured by plunging a needle into it. Despite the operation the process of gastrulation still took place in most cases, and a normal embryo developed. In consequence of the operation, as sections show, a large part of the fluid of the blastocœl is set free, although a small part of it may remain. When the operation is carried out at an early stage the cavity may develop later and suffice to bring about the gastrulation, if it were really due to this factor. In order to meet this possible objection, I operated on two other sets of eggs, one at the time when the

blastocœl had reached its highest point of development, and the other at the time when the dorsal lip of the blastopore had just appeared on the surface. Sections showed that the blastocœl was emptied in large part, yet in both cases gastrulation took place. There is, however, to be noticed a distinct retardation in the time of gastrulating between the normal and the injured eggs. The hole made in the roof closes almost at once, but a lump of cells generally indicates, throughout the gastrulation process, the place of injury. Owing to the closure of the top, the pressure relations of the cells will be again largely reëstablished, but the delay in the time of gastrulation indicates that the injury has to some extent interfered with the processes involved in the act of gastrulating.

The results show that Rhumbler's hypothesis is probably incorrect at least so far as the blastocœl is concerned. If however he should shift his position and assume that it is the accumulation of waste substances in the interior of the egg itself, *i. e.*, in the middle of the yolk-cells, that causes the invagination, the results are not fatal to his view. I attempted therefore to test Rhumbler's hypothesis in another way. If the amount of carbon dioxide outside the egg can be made equal to that in the interior, the process of gastrulation should not occur, if Rhumbler's view is correct. I placed frog's eggs in the late blastula stages in a bottle containing some water. By means of a tube running beneath the water I forced air that I had held in my lungs for a few seconds through this water. The air above the water was also displaced through an outlet. The communications with the outside were then closed. It seemed probable that there would be as much, and probably a great deal more carbon dioxide outside the eggs than inside, yet during the following six to twelve hours the gastrulation took place in the normal manner. This result also is not favorable to Rhumbler's interpretation. Other observations and experiments have led me to think that the process of gastrulation cannot be explained by such mechanical processes as surface tension, and I have tried to show elsewhere [1] that the change in shape of the cells that leads to the invagination is due to a process of active contraction of the cells, which

[1] *Raux's Archiv*, XIX., 1905.

in turn is the outcome of the pressure relations of the cells on each other.

Effects of Cold on the Early Development.[1]

In a preceding paper[1] I have described some abnormal embryos of the frog that were produced as the result of cold. The early segmentation stages of these eggs were not preserved, but I can now make good this deficiency by means of a series of young stages kept under similar condition (1° to 2° C.), that have been put up for me by Dr. N. M. Stevens.

The most obvious effect of the cold is of course to delay the development. The secondary effects involve (1) greater injury to the yolk cells than to those of the upper hemisphere, (2) effects on the formation of the segmentation cavity, and (3) in some cases the retention of the small cells at the upper hemisphere. How far this latter change may be directly connected with the injury to the lower cells, so that they fail to draw inwards, is a point difficult to determine, but if the cold is responsible for the failure of these cells to draw inwards, the retention of the small cells at the upper pole may directly result.

The details of the development of these eggs, left on the ice for eleven days, are as follows (the eggs were put on the ice April 9, at 10 a. m.):

April 9, 1 p. m. Some eggs still in the four-cell stage, others going into eight cells.

April 9, 6 p. m. Eight-cell stage. Small segmentation cavity present.

April 10, 9 a. m. Some eggs going into the twelve-cell stage. The segmentation cavity is well developed (Fig. 18).

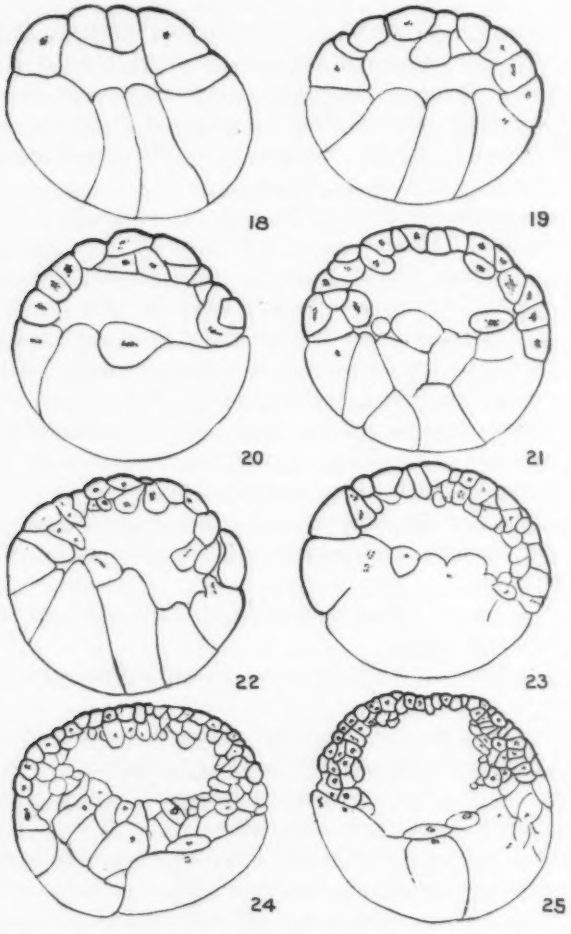
April 10, 5 p. m. Sixteen-cell stage. The segmentation cavity extends far down into the yolk.

April 11, 9 a. m. Another cell division has taken place. A large segmention cavity has developed (Fig. 19).

April 11, 8 p. m. Immigration of cells at the top of the egg taking place. Segmentation cavity better developed on one side than on the other.

[1] *Roux's Archiv*, XIX., 1905.

April 12, 9 a. m. Further division has occurred. Black cells are noticeably confined to the upper third of the egg, *i. e.*, there is not the normal downgrowth (Fig. 20).



FIGS. 18–25. Segmentation stages of egg kept on ice. *Rana palustris.*

April 12, 6 p. m. Condition same as last. Segmentation cavity large.

April 13, 9 a. m. Same condition; possibly another division has occurred.

April 13, 6 p. m. Some of the eggs have divided further. Large blastocœl (Fig. 21).

April 14, 9 a. m. Same as last.

April 14, 5:15 p. m. A large and irregular segmentation cavity, lying more on one side, is seen in sections (Fig. 22).

April 15, 9 a. m. The surface of the egg shows white flecks, and the cells have divided further. Large blastocœl present.

April 16, 2 p. m. Same. Cells irregular. Segmentation cavity large.

April 18, 9 a. m. Same. Large segmentation cavity. No downgrowth of small cells. Yolk irregularly divided (Fig. 23).

April 20, 9 a. m. Divided further. Top of egg brown instead of black. Large and irregular segmentation cavity (Fig. 24).

April 22, 9 a. m. In the surface view the outlines of the upper dark cells are very irregular. Large segmentation cavity surrounded by irregular cells (Fig. 25).

Compared with the normal set,[1] put up at the same time, the retardation of this cold series is apparent. After eleven days these eggs on the ice did not develop further than those at room temperature in twenty-four hours. Not only is there a delay, but the development has become quite abnormal, especially during the later stages. The sections show that the cells have in general an irregular outline, and are not compacted together as in the normal embryo. There is no downward migration of the material of the top of the egg, hence the abnormal forms of embryos described in my former paper. Great differences and abnormalities are found in the formation of the segmentation cavity, and this same difference was noticeable in the older stages. These older stages have been described in my earlier papers.

The Early Development of Lithium Larvæ of the Frog.

In a previous paper[2] I have described some of the effects of lithium solutions on the development of the frog's egg. The

[1] The control normal set are those described in my forthcoming paper on "The Origin of the Embryo-Forming Materials in the Frog's Embryo," 1906.

[2] *Roux's Archiv*, XVI., 1903.

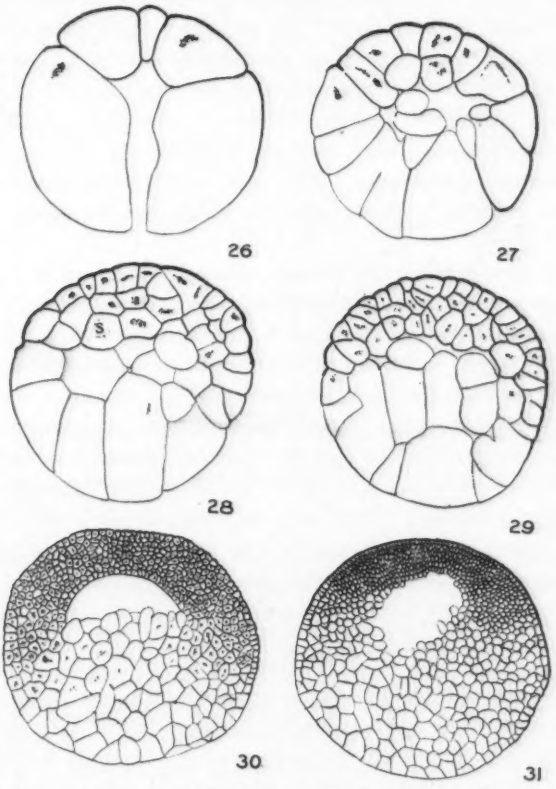
most peculiar and interesting type of these larvæ is that in which the embryo is formed in the interior of the egg instead of on the surface. In other cases the material of the top of the egg accumulates as a solid black cap upon which the anterior end of the neural plate sometimes appears. Embryos also appear in which the dark cells extend to different distances over the yolk.

The material that I used to study these embryos lacked the earlier stages, and while the observations on the living eggs left little doubt that the results were due to the failure of the upper cells to move downwards over the yolk, yet the actual details were not known. Since the question of the origin of the embryo-forming material is involved in my interpretation of the results it was desirable to obtain these missing stages. Dr. N. M. Stevens has kindly put up for me the necessary material for studying these missing stages of *Rana palustris*, the same species that I formerly used.

The eggs in the two-cell stage were put into a 0.5 per cent. solution of lithium chlorid and preserved at intervals of two hours. After the first two hours the eggs were in the eight-cell stage. A section through one of these is represented in Fig. 26. It differs little from a normal egg.[1] The segmentation cavity is somewhat smaller than that of the normal egg at this time. Two hours later the egg had reached the condition shown in Fig. 27. The inpulling of the cells of the upper hemisphere has begun. The segmentation cavity is noticeably small. Two hours later, Fig. 28, the divisions have gone further, and the upper cells now form almost a solid cap around the upper pole. A very small segmentation cavity, *S*, is present. Two hours later, Fig. 29, the smaller cells at the top still remain in place. A flattened segmentation cavity is present, but much reduced as compared with that of the normal egg. During the next four hours the changes are not marked. The small cells remain at the top and the segmentation cavity remains small. Two hours after this, *i. e.*, six hours after the last stage figured, the eggs are in the condition represented in Fig. 30. The roof of the segmentation cavity is very thick and the small cells still remain in the upper hemisphere. Four hours later, Fig. 31, the material at the top of the

[1] In preserving the lower blastomeres became separated in the egg.

egg has concentrated in a cap. It represents all of the ectoderm of the embryo. This brings the development to the stage at which my former series begins. If, after this stage is reached, the ectodermal material at the top of the egg turns into the interior, instead of growing down over the surface, as in the normal egg, an invaginated embryo is formed.



FIGS. 26–31. Segmentation stages of egg in lithium chloride. *Rana palustris.*

If it fails to turn in, it remains as a solid cap at the top, and if not too condensed the anterior end of the neural plate develops.

In the lithium types there is rather a sharp line of demarcation between ectoderm, mesoderm and endoderm. The three layers

appear to be more or less stratified in the order just given. This is best seen in the inverted type, in which the ectoderm turns in at the top. The mesoderm forms a sheet of cells wrapped around the neural plate, and the endoderm is drawn upwards as a sheet of cells over the mesoderm. (See Fig. I, Pl. XXIV., in my paper of 1903).[1] The endodermal layer, that is drawn upwards, appears to be that which is normally turned in around the lips of the circular blastopores. In addition to this there is always formed a tube opening on the surface that is lined by yolk cells. The opening of this tube lies near the equator of the egg and forms a crescent-shaped mouth there. The cells that line this tube correspond, I think, to those that make the anterior end of the normal archenteron. This part of the archenteron of the normal embryo appears to develop by the drawing apart of the yolk cells.

The mesoderm of the lithium larvæ is made up of the cells that lie just beneath the ectodermal cap. If we may draw any conclusion from the condition of the mesoderm in the lithium forms in regard to the origin of the first formed mesoderm of the normal embryo, we would conclude that it does not come from those cells of the 32- to 64-cell stages that are drawn beneath the surface at the top of the egg, but from cells lower down at about the level of the upper portions of the lower four blastomeres of the eight-cell stage. There may be, however, not a little latitude in respect to the potency of many of these cells, and their location is probably also a factor in their differentiation.

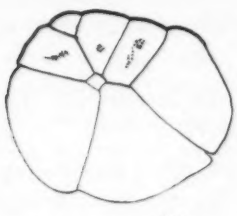
Effects of Lithium Chlorid and Sodium Chlorid Acting Together.

The effect of lithium seems to depend in large part upon chemical, rather than upon osmotic, effects. Other salts also act chemically in somewhat the same way as lithium, but to a less degree. I have tried the effects of these salts acting together and also separately on the eggs of *Rana palustris* and of *Bufo lentiginosus*. The material for the former species I owe to Dr. Stevens.

A solution of LiCl 0.5 and NaCl 0.6 per cent. gave the results shown in Figs. 32 and 33. The cleavage was not only greatly

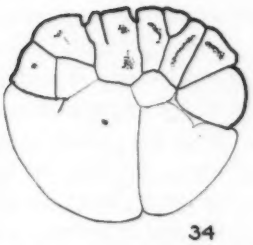
[1] *Roux's Archiv*, XVI.

retarded but more irregular than normal, and came to an end before many divisions had been carried out. The details of the results were as follows: —

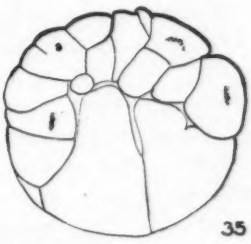


32

33



34



35

FIGS. 32–33. Segmentation of egg in lithium chloride and sodium chloride. *Rana palustris.*

FIGS. 34–35. Segmentation of egg in lithium chloride and magnesium chloride. *Rana palustris.*

The eggs in the two-cell stage were put into the double solution. After two hours a normal looking two-cell stage was present. After another two hours the cells had divided, and were in the same condition two hours later, Fig. 32. The egg shows little or no segmentation cavity. Other eggs have an abnormally small segmentation cavity. After two hours more, the upper cells have again divided, and the upper ends of the lower cells also. After two hours more, the eggs appear to be in the same condition, and this is also true for the next six hours. Sections of these eggs show a tendency for the blastomeres to run together (Fig. 33), and each contains several nuclei. After a further interval of eight hours, the cell-walls have largely disappeared on the surface.

The results show very clearly that the double solution has acted more injuriously on the egg than the lithium chlorid alone. The results appear, from a comparison with sugar solutions, not to be entirely due to the increase in osmotic pressure, but to the greater effect of the combined action of the sodium and the lithium.

Effects of Lithium Chlorid and Magnesium Chlorid Acting Together.

Eggs in the two-cell stage were put into a solution containing LiCl 0.5 + $MgCl_2$ 0.6 per cent. After two hours the eggs were still in the two-cell stage. Two hours later the eggs were in the 16-cell stage, some of them having divided with great irregularity. Sections show a small segmentation cavity present. Two hours later, Figs. 34 and 35, one or two further divisions have taken place. As shown in these figures the egg is nearly solid, and in other eggs there is no trace of the segmentation cavity. Another division has taken place after two hours more, and another division after another two hours. At this time the sections show the interior cell-walls running together to produce polynuclear cells. Two hours later the egg is as before, or has divided again, and after another four hours further division has occurred, but in this and the following stages (after eight hours), the cells especially the yolk cells are becoming polynuclear and division is apparently near its end without having produced a large number of cells. It is noticeable that the yolk cells have been more affected than the smaller cells of the upper hemisphere. The results are similar on the whole to those with LiCl + NaCl, but less marked; the younger stages being more regular. The segmentation cavity appears to have been practically obliterated.

The Chemical versus the Osmotic Effects of the Salt Solutions.

In order to determine, if possible, how far the action of the salt solutions is due to chemical effects and how far to osmotic effects, the following experiments were carried out with the eggs of *Rana sylvatica*. Eggs in the two-cell stage were put into solutions

of graded strengths. The amount of salt is given in percentages although this is not quite accurate, since, in the first place, one per cent., for example, is one gram added to 100 cc. (and not to 99 cc.) of water; and in the second place a certain further decrease in strength is caused by the water in the jelly around the eggs.

It is not an easy matter to determine definitely where normal development ceases, for, not only is the development often stopped at different stages in the same solution, but there is also a considerable overlapping in the different solutions. I have arbitrarily selected as the upper limit, the strength of solution in which, although the cleavage may continue for a time, gastrulation does not occur.

For LiCl alone it was found that gastrulation may take place and a normal embryo appear in a 0.5 per cent. solution. In a 0.55 per cent. solution the blastopore is large, its closure is delayed, and even prevented. In a 0.6 per cent. solution the blastopore appears, but generally no embryo develops; while the upper limit seems to be about 0.65 per cent. where practically all of the eggs fail to pass beyond the late segmentation stages. For NaCl the upper limit is above 2.0; but owing to an accident the upper limit was not accurately determined.

In a solution of LiCl 0.4 per cent. + NaCl 0.3 per cent. only the late segmentation stages appeared. Thus both solutions, being lower than the maximum for each, produce an injurious effect. In another double solution of LiCl 0.5 per cent. + NaCl 0.5 per cent. a few nearly normal embryos developed, but in a solution of LiCl 0.5 per cent. + NaCl 1.0 per cent. only the late segmentation stages developed, or rather a cap of black cells appeared at the top of the egg (surrounded in some cases by a band of gray cells, as in lithium larvæ that I formerly described). In a solution of LiCl 0.5 per cent. + NaCl 1.5 per cent. only late segmentation stages developed. This then may be taken as the limit.

A solution of cane sugar must be quite strong in order to prevent the development of the embryo. Even a 6.0 per cent. solution gives normal embryos. An 8 per cent. delays the closure of the blastopore, and a 10 per cent., while not preventing the appearance of the blastopore, does prevent its closure,

and no embryo develops. In a 12 per cent. solution the egg reaches only the late cleavage stages.

In a solution of LiCl 0.4 per cent. + sugar 5.0 per cent. only about the sixty-fourth-cell stage is reached. Thus together the two substances produce an effect that neither alone affects. In another case, LiCl 0.5 per cent. + sugar 1.0 per cent. delayed the closure of the blastopore; a solution of LiCl 0.5 per cent. + sugar 2.0 per cent. also delayed, but did not actually prevent the closure of the blastopore; while a solution of LiCl 0.5 per cent. + sugar 4.0 per cent. stopped the development of most of the eggs in the late cleavage; although in one case at least the outline of the neural plate appeared. The results show that the sugar reinforces the effects (or a part of it at least) of the lithium. It remains now to see whether the results can be explained as due to osmotic pressure alone or whether a chemical effect also occurs.

Concentration.		*Ionization.*	*Osmotic Pressure at 18° C. in Atmospheres.*
Per Cent.	Mols. Per Liter.	Per Cent.	
Cane Sugar.			
1	0.029	0	0.698
2	0.058		1.396
4	0.117		2.792
6	0.175		4.188
10	0.292		6.980
12	0.351		8.376
Lithium chlorid.			
0.4	0.094	82.3	3.843
0.5	0.118	82.3	4.804
0.55	0.129	82.3	5.284
0.6	0.141	79.9	5.690
0.65	0.153	79.9	6.161
Sodium chlorid.			
0.3	0.051	87.4	2.15
0.5	0.085	85.2	3.55
1.0	0.171	81.8	6.96
1.5	0.256	79.1	10.28
2	0.342	77.7	13.61
3	0.513	73.7	19.95
4	0.684	71.6	26.28
Lithium chlorid + Sodium chlorid.			
0.4	0.3		5.99
0.5	0.5		8.35
0.5	1.0		11.76
Lithium chlorid + Sugar.			
0.4	5.0		7.33
0.5	1.0		5.50
0.5	4.0		7.59

In the accompanying table, kindly prepared by Dr. H. W. Berg, the osmotic pressures of most of the solutions given in the preceding statement are given. It will be seen for the upper limit of LiCl, namely, 0.65 per cent., the osmotic pressure is 6.161. In comparison the results with NaCl are very different. The upper limit is above 2 per cent. The osmotic pressure for 2 per cent. is 13.61, which is more than double the strength of effective LiCl. The comparison shows that the effects of the lithium salt are not due to osmotic pressure alone.

In a double solution containing LiCl 0.5 + NaCl 0.5 nearly normal embryos appeared, and the osmotic pressure in this case is 8.33, which is much greater than that for the lithium alone. It took, in fact, a solution containing LiCl 0.5 + NaCl 1.0 to prevent gastrulation. The osmotic pressure in this case is 11.76, a pressure far greater than that for the effective limit of LiCl alone.

The upper limit for cane sugar was found to be about 12 per cent., which corresponds to an osmotic pressure of 8.376. This is much higher than for the lithium chlorid alone.

For solutions of LiCl 0.4 + sugar 5.0 per cent., only the 64-cell stage was reached. The osmotic pressure is 7.33, which is again much higher than for lithium chloride alone, but less than that for the sugar alone. It would seem, therefore, that the two together must have a higher pressure than for the one producing its effects at the lower limit, but less than for the other that produces its effects at a higher pressure. Similar conclusions might be drawn from the double solution containing LiCl 0.5 + sugar 4.0 per cent. The results show that there is a double effect produced by salt solutions, a chemical and an osmotic. How each effect is produced we do not know at present. Loeb has shown, however, that living substances behave very differently towards the amount of water absorbed according to what chemical element, sodium, calcium, or lithium, for example, that they have taken up. Since the development of the embryo is associated with the amount of water absorbed it might appear that in this way the chemical action is similar to the results produced by means of osmotic pressures which also effect the amount of water. That the egg contains enough water in itself for normal development is shown by my

experiments of causing it to develop out of water, but this does not show that if water is removed beyond a certain point or the capacity to absorb water is changed the development might not be delayed. · In fact if the egg is dried too much it fails to develop. However this may be, the point of special interest brought out by these experiments is that an effect may be produced by a double solution in which the total osmotic pressure is lower than that required to produce the effect by one of the substances alone, but higher than that sufficient to produce the result by the other alone. It seems probable that the effect is a double one ; in part chemical, in part osmotic.

OBSERVATIONS ON THE REACTIONS OF CRYPTOBRANCHUS AND NECTURUS TO LIGHT AND HEAT.

A. M. REESE.

During most of the time for the past four years the writer has had one or more specimens of the giant salamander (*C. alleghenensis*) in captivity in the laboratory, and the tendency of these animals to seek the darker parts of the tanks in which they were confined, or to crawl under any sufficiently large object that might be present, led to the experiments which are summarized in the present paper.

The experiments with *Necturus* were performed on five individuals of average size, though most of the light experiments were carried on upon one individual.

Four large specimens of *Cryptobranchus* were used for both sets of experiments, the heat experiments being nearly all performed after the completion of the light experiments.

In the light experiments three sources of illumination were employed: A sixteen candle-power incandescent electric lamp, so shaded that the light could be thrown on any given part of the animal without illuminating the rest of the tank; the direct rays of the sun, reflected from a small mirror; and an ordinary arc, projection lantern, which was set up at the side of the tank, so that a narrow beam of light could be reflected from a mirror into the water.

In the color experiments red and blue globes were used with the incandescent lights, and with the other two methods of illumination plates of red and blue glass were introduced into the white rays from the sun or from the electric arc.

It was found, by the use of the spectroscope, that the red plate gave an almost pure red, while the red bulb gave, besides the red, some yellow and green rays. The blue plate gave the entire spectrum except the dark green, yellow and yellowish red; the blue bulb gave the entire spectrum, which apparently differed

from that of the ordinary white bulb only in intensity. Owing to this impurity in the colors, especially the blue, the observations with the red and blue lights cannot be given much weight; some of the observations will, however, be briefly given below.

It was found that both with *Cryptobranchus* and *Necturus* the responses to light were much more marked for the first ten or a dozen stimulations than for succeeding stimulations, so that it was necessary, on account of this loss of sensitiveness, to make only a comparatively short series of experiments at any one time. Ordinarily these animals will lie for many minutes or, possibly, even hours without the slightest motion, so that their reaction to stimuli at these times is too evident to doubt; but occasionally, especially at night, they become restless, and it becomes necessary to postpone experimentation. In each case where no reaction was obtained within two and one half minutes the reaction was recorded as "none." Since the animals under observation were at all times covered with several inches of water it seemed unnecessary to use any form of heat screen, as this depth of water would absorb all heat rays from the artificial lights if not from the solar illumination.

Reactions of Cryptobranchus to Light.

The effect of a ray of white light when thrown on different parts of the body was first tried. It was found that all parts of the body are sensitive to white light, but that the tail is by far the most sensitive region. While enough experiments were performed to show that the middle regions were more or less sensitive to white rays, it was the head and tail that were chiefly studied in their reactions to light stimulation.

When a ray of white light was thrown upon the tail the response was, in very many cases, immediate, and consisted in a quick forward movement of the animal until the tail was removed from the illumined area. In almost no cases among many trials was the response delayed for as much as a quarter of a minute, the average time for a response being about three seconds or less. The extreme sensitiveness of the tail of this animal to light stimulation is quite remarkable, and the response is, in all cases, exactly the same.

In about half of the experiments where light was thrown upon the head of *Cryptobranchus* no response was obtained even at the end of two and a half minutes or longer, and in no case was a response obtained in less than nine seconds: however, sufficient undoubted responses were obtained to justify the statement that the head of the animal is sensitive to white light. The response in this case, when it came at all, was invariably shown by the animal's backing away from the light.

The responses were about as sudden and as strong when the incandescent light was used as when the much stronger illuminations were employed; this may have been due to the fact that when the rays from the sun or the arc light were used the tank was partially illumined by diffused light, while in the other case there was no light except the small circle that came from the shaded incandescent bulb.

That *Cryptobranchus* is sensitive to white light of even weak intensity seems proven by the fact that the animals seek the darker parts of their tank in ordinary diffused light.

The results obtained with the red and blue lights were not so definite as those with the white light, and, as has been previously stated, may have but little value. With the red plate which gave, with the arc light, a strong, pure red illumination, no responses at all were obtained, either from head or tail stimulation. The incandescent light with the ruby globe, while not so strong a light, produced, in most cases, decided responses; this was possibly due to the fact that the light was not nearly so pure a red as that given by the plate. The responses were of the same character as with the white light, the difference being one of rapidity.

In no case was the response to a stimulus instantaneous, as with the white light, but in several instances reaction took place, when the tail was stimulated, within two seconds. As with the white light, the head was much less sensitive than the tail.

The reactions to blue light were of the same character as with the other forms of illumination, but were more rapid than with the red light. The apparently greater sensitiveness to blue than to red light may have been due simply to the greater purity of the latter color.

Reactions of Necturus to Light.

The same kinds of experiments were tried with *Necturus* as with *Cryptobranchus*. All parts of the body seemed sensitive to white light, but in this case the head was more sensitive than the tail, the withdrawal from the circle of illumination being instantaneous, in many cases.

The effect of illumination from below was also tried with *Necturus*, the animals being placed in a glass aquarium which was shaded from above, while a beam of sunlight was thrown on the ventral regions of the body. All parts of the ventral surface were sensitive to this form of stimulation, but the head was, in this case, much less sensitive than the tail. The responses as a whole were neither so quick nor so strong as when the light fell upon the dorsal regions of the body.

A beam of red light produced by the same red plate that was used in the experiments upon *Cryptobranchus* produced no reactions that were definite enough to be of value. The red, incandescent bulb, on the other hand, caused fairly strong reactions, those from the head and tail being of about the same suddenness.

The reactions to blue light, whether produced by the plate or the incandescent bulb, were much more decided than those to red light; the average reaction time for the head and for the tail was about the same.

No experiments were tried to determine the effect of red or blue light upon the ventral side of the body, either with *Cryptobranchus* or with *Necturus*.

That it is not the organs of the lateral line system that respond to these light stimuli seems probable, at least in *Cryptobranchus*, from the fact that the head region, which is most abundantly supplied with the organs, is less sensitive to light than is the tail. In *Necturus*, when the light came from above, the head gave the most sudden responses; but when the light was from below the tail was more responsive than the head. This would seem to indicate that the more rapid response from the head was due to the sensitiveness of the eyes, though why this should be the case with *Necturus* and not with *Cryptobranchus* is not apparent.

Parker ('05) thinks that the sensitiveness to light of young lamprey eels is due to stimulation of the ends of the spinal nerves in the skin; this may be the case here also. He found ('05) that in the ammocœtes, as in *Cryptobranchus*, the tail is the most sensitive part of the body, the response being negative. In the frog, on the other hand, he found ('03) the reaction to be positive. Just as it is of value to the young lamprey, on account of its burrowing habits, to have a tail that is sensitive to light, so is the sensitive tail probably of value to the giant salamander because of the animal's habit of concealing itself under objects in its native haunts. The fact, as I have already shown ('05), that the eyes of this animal are probably not very sensitive, may have something to do with the unusual sensitiveness of the tail.

Thermic Reactions of Cryptobranchus.

The temperature experiments were made upon four adult specimens, the same ones that were used in the light experiments.

When taken from their tank, where the water was at 18° C., and put into water at 33° none of the four animals showed any signs of being aware of the change of medium; the same negative results were noted when they were changed from water at 5° to water at 26°. On one occasion, however, they showed slight signs of discomfort when put from water at 14° into water at 26°.

Removal of the animals from water at either 18° or 26° to water at 42° resulted in the most violent struggles, beginning after an immersion of two or three seconds and lasting, usually, for a minute or more, or until the animal was completely exhausted. The violence of these struggles was quite remarkable, though less marked in some cases than in others. In some instances the struggles continued until the animal was completely exhausted and turned belly side up as though dead; in other cases the struggles gradually ceased until the animal lay quietly in the warm water. Two of the animals died soon after the experiments, probably as the result of their experiences, as they had seemed perfectly healthy before.

Removal from water at 26° to water at 5°, or from water at 18° to water 0° caused no reaction, although, in the former case,

there was a decrease in temperature of 21°. Neither did an increase of 21° or more cause any reaction unless the higher temperature reached 40° or more. It would seem, then, that *Cryptobranchus* is not sensitive to considerable changes in temperature, but is very seriously affected by a temperature only slightly higher than that of the human blood.

Thermic Reactions of Necturus.

As might be expected, perhaps, from its having gills, *Necturus* proved to be more sensitive to temperature variations than was *Crytobranchus*.

When transferred from water at 0° C. to water at 18° three of the five animals showed little or no reaction, but the other two darted about the tank in a curious, spasmodic way quite different from their usual motions. When transferred from water at 18° to water at 32°, three of the animals again showed but little activity, while the other two struggled quite violently. When put into water at 42° from water at 32° or lower, all of the animals struggled as violently as did *Crytobranchus*.

When put into water at 4° from water at 32° two of the animals again showed marked activity, while the other three were scarcely affected. A very short stay in the warm water was sufficient to completely exhaust all of the *Necturus*, so that they turned belly side up, but when returned to water of moderate temperature they soon recovered.

Necturus is apparently sensitive to considerable changes in temperature, both rising and falling, as well as to the higher temperatures to which *Crytobranchus* responded.

Other and more accurate experiments along these lines suggested themselves, but on account of the lack of the necessary facilities they had to be postponed.

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NOTES ON BRANCHIOBDELLA.

W. M. SMALLWOOD.

So little is known concerning the American members of *Branchiobdella* that the following notes may be of interest. During the summer of 1905 a number of crayfish were taken by dredging from Lake Clear, Harrietstown, Franklin County, N. Y., in about eighteen feet of water. On all of the claws the carapace, rostrum, and even on the abdomen there were large numbers of *Branchiobdella instabilia* Moore which Professor Moore has kindly identified for me. I found associated with them a few *Branchiobdella pulcherrima* and a half dozen *Bdellodrilus illuminatus*.

All of these are usually designated as external parasites but I can see no reason for using this term. The word symbiosis or commensalism more correctly describes the relation that exists between the crayfish and *Branchiobdella;* but these two terms are used with such inconsistency to-day that we can find no accepted definition of either. It is not easy to conceive how *Branchiobdella* is of benefit to the crab. Their whitish appearance and constant movements make the crab more conspicuous yet from their location and the fact that they are attached by the posterior end suggest the probability that they do not receive anything as food from the crab's body.

The food of these forms throws some light on their habits. A study of the digestive tube in both macerated and mounted sections revealed the presence of large numbers of unicellular algæ and diatoms. The digestive tube was full and seemed to contain nothing but small plants which would suggest that the crayfish merely served to carry the worms around enabling them the better to secure food so that it seems to me that the term symbiosis used in its original and etymological sense gives a better idea of these relations than does external parasite.

All of these symbionts are continually in motion, stretching the body out in every direction as if constantly feeling for something.

During these movements *B. instabilia* change their shape, the long, slender worm quickly becomes short and broad posteriorly. I had some small clepsine in the same aquarium and was much impressed with the great similarity of movement between the two. This peculiar leech-like movement together with the prominent posterior disk by means of which the animal adheres to the crab so simulates the general external appearance of leeches that it was doubtless one of the reasons for classifying *Branchiobdella* at first with the hirudineans.

Moore, '93, first describes these two representatives of *Branchiobdella* and reports them only from Watauga Co., North Carolina, and Delaware Co., Pa., and so far as I have been able to determine they have not been recorded as occurring farther north than Pennsylvania. The water of Lake Clear is derived almost entirely from springs, but two mountain brooks flow into it, so that the water does not warm up much during the summer. The latter part of July, when the worms were taken, the temperature of the water along the shore rarely exceeded 70° F. during the day. When the crayfish having the parasites were placed in a small aquarium the *Branchiobdella* did not live more than twelve hours although the water was taken directly from the lake and changed once during that time. I am inclined to attribute their death largely to the rise in the temperature of the water as the aquarium was placed in the boat house, which was at least 15° to 20° warmer than the water in the lake.

The animals taken all seemed to be sexually mature, as subsequent study proved, and were all about the same size. Although I have over fifty specimens of *Branchiobdella instabilia* there does not seem to be any noticeable variation in their size or appearance.

The biannulation of all but the head segment in *B. pulcherrima* and the first four just back of the head in *B. instabilia* is very suggestive of leech-like affinities in these forms, which are now generally agreed as being oligochætes. The cœlomic cavity is clearly defined and perfectly divided by dissepiments into a series of cavities. The cœlomic space is encroached upon by the enormously long muscle cells which correspond to the well-known circular and longitudinal muscles of the oligochætes.

After studying a considerable number of animals in serial sections I find but one type of muscle cell. The contractile tissue is arranged around the outside of the cell and is striated in most instances. Within the contractile region there is a granular protoplasm containing a spherical nucleus which corresponds to the ordinary nucleus found in any tissue. Voigt, '86, describes several arrangements for the contractile tissue in the European form *B. varians.*

The nervous system in both *B. instabilia* and *pulcherrima* can be easily seen in a mounted specimen and the ganglionic swellings in the latter make a very clear demonstration of their relation to somites. In section the nerve strand is seen to be clearly bilaterally symmetrical.

My chief observations are concerning the development of the sex cells and the results do not confirm all of the work done by Voigt. Voigt, '85, has given a full description of the formation of the eggs and sperms of the European *Branchiobdella varians* with 135 figures. The parts of the adult sperm as described by him are the same as those in *B. instabilia.* Concerning the development of the germ cells, however, the agreement is not so satisfactory.

The nebenkorper is unmistakably described for the nucleolus in the ovocyte on page 322. In Fig. 57 "Sind die kerne der spermatocyten noch ungeteilt; in Zweien von Zellen ist das Nebenkörperchen noch einfach, in den anderen bereits doppelt." In the figure referred to above and in succeeding ones there can be no doubt but that the nucleolus is the body described under the caption of Nebenkörperchen.

The spermatid has two nebenkörperchen, one that has persisted, the other is developed from the thicker protoplasm near the cell wall. "Das eine davon is das Nebenkörperchen, das andere besteht, wie sich herausgestellt hat aus einer Ausammlung von dichterem Protoplasma an der Zellwand, da, wo der Schwanzfaden hervorwachst und soll in folgenden als 'Bildungskörperchen des schwanzfaden' bezeichnet werden" (page 324).

In describing the parts of the adult spermatozoön the nebenkern is derived from the nebenkörperchen, and, as already cited, this latter structure persists and becomes the tip end of the

sperm. The parts of the sperm and their derivation are summarized in the following words: "Der Samenkörper besteht also jetzt (Fig. 132): (1) aus dem nebenkern, (2) dem von einer dünnen Membran gebildeten Schlauch, (3) dem asu dem Kern hervorgegangenen konischen Teil, (4) dem Verbindungsstück, welches aus dem protplasma der Zelle anstand und aus welchem, (5) der Schwanzfaden hervorgesprosst ist," page 328.

Moore, '95, describes the morphology and histology of *Bdellodrilus* in detail, a form which was first described as *Branchiobdella*, and the agreement of *B. instabilia* is so close that a separate description is not warranted. In connection with the description of the reproductive system the following statement is made: "The first steps in the development of spermatozoa begin before the worm has nearly reached full size, and proceeds continuously; the various stages floating freely in the cœlom, in which they complete their development. In the mature worm the cavities of the fifth and sixth post-cephalic somites are filled with spermatozoa in various stages of development, while the testes proper have become much reduced and inconspicuous. The details of this process have been admirably worked out and described by Voigt; and it need only be added that what observations the writer has made are in accordance with his account" (page 519). In the same year Calkins' paper appeared on the spermatogenesis of *Lumbricus* and makes no mention of the works of Voigt.

The ovaries are located in the seventh post-cephalic somite and consist of two separate sacs, one on each side of the digestive tube. The young ova in one half of the ovary may be in a state of division while the rest of the ova are in the resting state. This division is by the usual indirect process with no evidence of astral fibers. A distinct centrosome is present at each pole of the spindle, the spindle fibers arising from within the nucleus. The chromatin assumes the characteristic spireme state preceding the formation of the equatorial plate. An early prophase stage in the division of an ovum is shown in Fig. 5. Voigt figures ova having two nucleoli and believes that this is an indication of amitotic division. My sections have been studied with this particular point in mind and no case of amitosis has been noted. In a few

instances ova containing two nucleoli (Fig. 4) were found. The nucleolus is large and when mitosis begins has undergone a transformation similar to the changes described for *Haminea* (Smallwood, '05). This would indicate that the nucleolus breaks up and does not take an active part in division. Furthermore, in some cells the two centrosomes in the prophase were observed while the nucleolus was still present, the difference in size is alone sufficient to prevent confusion; Voigt has apparently confused them in describing the process of direct division.

As the eggs mature, they are found on the surface of the ovary and can be readily distinguished by their large size as compared with the surrounding young ova. Associated with the ovary on one side only in most instances, there is found a large mass which is apparently nutritive in character (Fig. 3). An attempt was made to detect the beginning of this modification. The membrane which surrounds the ovary is continuous and passes around the nutritive mass. This structure extends from the extreme dorsal part of the cœlome of the seventh somite to the

FIG. 1. A photograph of *Branchiobdella instabilia* contracted.

ventral body wall (Fig. 3). Several sections show this mass even passing beneath the intestine. Frequently the neural sheath of peritoneum can be shown to be continuous with the sac enclosing this mass and the eggs. All of the masses studied had a faint nucleus and in one instance a nucleolus was found which would

seem to indicate that this mass was a very much enlarged cell. One animal showed a few small ova in the edge of this mass adjacent to the ovary but in all other animals there was a clearly defined outline between the ova and this mass, which indicated unmistakably that the ova cells were in the ovary. This nutritive mass shows two clearly defined regions, a dorsal and ventral. The dorsal portion contains many small round bodies very uniform in size in a protoplasmic matrix. These bodies take a basic stain while Bordeaux red colors the matrix; the ventral region does not contain these bodies, nor is it composed of granular protoplasm but yet it takes a basic stain. Its appearance in the fixed state suggests that it exists as fluid in the living animal. When the egg of the hydroid *Clava* is stained as above and viewed with the oil immersion lens, the conditions are so nearly identical that unless one knew in advance I very much doubt his ability to distinguish the two as belonging to different species. It is interesting to find this similarity in two such widely separated animals and it also helps to interpret the conditions in *Branchiobdella instabilia*. These bodies are interpreted as nutritive by Hargitt,[1] probably of a proteid nature. In *Branchiobdella*, I am inclined to believe that this nutritive mass is discharged into the cocoon where it nourishes the growing worms.

Voigt finds the beginning of what he designates a degeneration process. This process consists in the deposition of fat-bodies in certain ova but no such large size is reported nor is the change limited to one cell. Of course one can not be sure that but one cell has taken part in the formation of this large nutritive cell for the process may involve the growth of one cell at the expense of many others as is so often the case in the growth of eggs. But that this change is a fat-forming process is much to be doubted as no fat reactions were obtained. It is probably rather a normal growth process, the nutrition being stored in this special cell rather than in the cytoplasm of each egg.

What has already been said for the appearance of the ovary and manner of division may be repeated for the cells in the testes. In size, shape, reaction to stain, and appearance, they are

[1] Hargitt, C. W., "The Organization and Early Development of the Egg of Clava leptostyla," in press, BIOL. BULL.

very similar. The testes are usually somewhat larger than the ovaries and contain more cells. They are located in the sixth post-cephalic somite. Frequently the cells in the testis are pear-shaped as contrasted with the spherical form of the young ova.

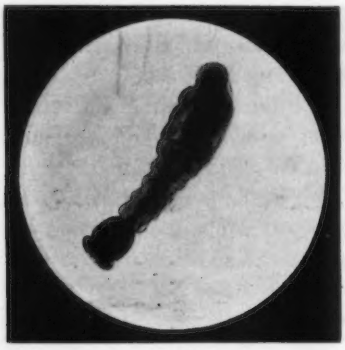

FIG. 2. A photograph of *Branchiobdella pulcherrima* showing the post-cephalic segments, the digestive tube and ganglionic swellings. In the sixth post-cephalic segment the two testes are on one side and in the seventh the ovary shows.

No evidence was observed to show that nutritive bodies such as Voigt reports for *B. varians* were present in the cytoplasm; nor is there a special nutritive cell as in the case of the ovary. In *Branchiobdella pulcherrima* the two testes were found on the same side in one animal (Fig. 2).

Associated with the formation of the male sex cells from the time they leave the testes until the spermatozoön becomes fully grown there is a protoplasmic structure, termed the blastophore (Bloomfield, '80, Calkins, '95) in *Lumbricus*, and the cytophore (Voigt, '85) in *Branchiobdella varians*. These two terms seem to refer to the same structure and the more recent term, blastophore, will be used in this paper.

Calkins traces the formation of the blastophore and finds that it appears before the sperm cells leave the testis. While still in the testis he figures the blastophore as consisting of eight nuclei in an undifferentiated protoplasm. Repeated division of the nucleus unaccompanied by the formation of cells gives rise to a multinucleate cell. After a time "cytoplasmic cleavage occurs

around each nucleus, thus differentiating the blastophore from the germ cells" (p. 275). In *B. varians* (Voigt, p. 314 sq.) the blastophore arises in a similar although not identical manner. It is first seen just as the spermatogonia escape from the testis at which time but two or four cells are associated with it. When the spermatogonia divide, a portion of the protoplasm is not enclosed within the cell wall, although it is directly connected with the cytoplasm of the cell. The young stages show the blastophore more or less irregular in shape, but as the cells become more numerous it is uniformly spherical. It differs in two particulars from the conditions in *Lumbricus*: (1) it is found in an earlier stage in the cœlome; and (2) the spermatogonia cells are always distinct in outline from the blastophore, while the earlier stage in *Lumbricus* is more like a syncytium.

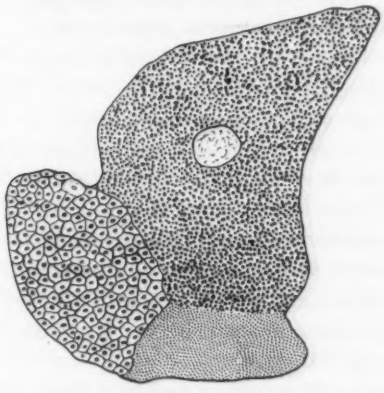

FIG. 3. A camera drawing of a single section of the ovary and nutritive mass. The dorsal granular portion contains a loosely organized nucleus. The ventral non-granular portion is larger in the other sections of it.

Branchiobdella instabilia repeats in the formation of the blastophore the conditions of *B. varians*. During the division of the spermatogonia the plane of division has a definite relation to the center of the blastophore. A definite spindle is always present with its long axis tangential to the surface of the blastophore, so that a plane passing through the equatorial plate, if continued, would go through the center of the blastophore. When the

spermatogonium divides, the division does not completely separate the two cells, but leaves them in connection with the blastophore. Each cell surrounding the blastophore has a direct protoplasmic connection with all of the other cells. Voigt finds as many as 128 cells surrounding the blastophore. One hundred cells were located with the camera lucida on the upper half of the blastophore of *Branchiobdella instabilia*, which would give about two hundred cells in all. The whole structure is so small that an accurate account is practically impossible. But from this estimate we can get some idea of the number of generations of spermatogonia and spermatocytes.

As the spermatid elongates, it remains attached to the blastophore by the tip end of the head and continues so connected until the spermatid has become a full-sized spermatozoön. The blastophore becomes reduced in size during this growth, vacuoles appear in it and the plasma stains give a more marked reaction. The reduction in size is so great in some instances that the blastophore is no larger than the leucocytes, but can be distinguished from them because of its non-nucleated condition. I believe that the blastophore is nutritive in character because of the origin and attachment of the developing spermatids and the accompanying degeneration in it as the spermatozoa approach full size (Calkins, p. 276), concludes his discussion of the blastophore with the following statement: "It lives as long as the developing germ cells are connected with it and dies when deserted by the spermatozoa. Nor is there any reason to suppose that it provides nutriment for the spermatid cell."

The phenomena of reduction are not readily determined in *Branchiobdella instabilia*. Continued search has been made to ascertain the number of chromosomes and at what stage the reduction occurred but thus far I have been unable to satisfy myself or to secure constant results. The size of the cells shown in Figs. 5 and 6 is the main reason for my failure. A study of the karyokinetic division of the cells surrounding the blastophore shows that the spindle fibers arise from the nucleus and terminate in a definite centrosome at each pole. No astral rays are present. The centrosomes lie close to the nuclear wall which gradually breaks down (Figs. 5 and 7).

As the spermatocytes become transformed into the spermatid, the chromatin gradually changes from the reticulate state into a compact, homogeneous mass. The spermatid is a small oval-shaped cell attached by the tip end of the head to the blastophore. Between the nucleus and the cell wall in the region farthest from the blastophore, there is a body which takes a basic

FIG. 4. A camera lucida drawing of an ovocyte with the No. 6 comp. ocular and 2 mm. objective.

FIG. 5. Prophase of young ovocyte drawn to the same magnification as Fig. 4.

FIG. 6. Metaphase of spermatocyte No. 12 comp. ocular and 2 mm. objective.

FIG. 7. Metaphase of spermatocyte showing the relation of the spindle to the nucleus, drawn to the same magnification as No. 4.

stain and is the centrosome or centrosomes of the last spermatocyte generation. The cells become so very small and the cytoplasm so much reduced that one can not be certain that there are two bodies; at this stage the body is much larger than the centrosomes during mitosis.

The distal portion of the cytoplasm becomes drawn out and forms the tail, at the same time the sperm centrosome elongates forming a connection between the nucleus and tail. This region becomes the middle piece in the adult sperm. The rapid elongation of the tail and nucleus soon renders it impossible to detect the presence of the cytoplasm. During the elongation of the nucleus, it stains more intensely and becomes constricted as if a large number of shallow parallel furrows crossed it at about 90° to the long axis of the sperm. The very tip of the sperm during nearly all of these changes remains in connection with the blastophore.

From the quotations cited from Voigt's interpretations of the spermatogenesis of the European *Branchiobdella*, it is apparent that there are important differences. In a few instances a structure which I regard as the nebenkern as described by Calkins (p. 289) was seen but this structure had no relation in its mode of origin to the nucleolus nor does it play an important rôle in

subsequent development. Voigt believes that he can trace the nebenkörpenchen from spermatogonium, through spermatocyte and spermatid to the apex of the sperm, which I very much doubt. What he has described and figured for the nebenkörpenchen in some of these early stages, I have frequently found in the free cells in the cœlome which are undoubtedly leucocytes. Kukenthal ('85, p. 454) who has made a study of the lymphoid cells of Annelids states that "The fluid of the cœlome is not homogeneous, but contains a number of elements of various kinds, among which the rather large rounded cells are the most conspicuous ; these are the lymphoid cells, and of them there are two kinds. Some have more or less finely granular protoplasm, and others contain clear highly refractive granules, which are colorless ; others are still larger, and of a yellowish-brown color." Voigt states that the nebenkörperchen can only be seen in the free living cells in some stages which is an additional confirmation of my criticism.

The statement that the spermatid has two nebenkörperchen, one of which is derived from the thickened protoplasm of the cell wall, is of course due to his inability to detect the origin of this body in the spermatocyte centrosome. The manner in which the nucleus is supposed to form the head of the sperm is somewhat unique. The head of the sperm grows out of the nucleus much as the radicle grows out of the bean, the nucleus becoming smaller as the head lengthens instead of the complete and simultaneous transformation of the whole nucleus into the head.

Branchiobdella instabilia is so closely related to *Branchiobdella varians* that the spermatogenesis of the two species is probably very nearly identical and a restudy of the latter will probably show its full agreement with the former as well as with other annelids.

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April 15, 1906.

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